

Listing of Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Currently Amended) A method for the treatment of a skin defect comprising
 - (a) culturing an intact hair follicle of an anagenic hair to obtain outer root sheath cells;
 - (b) culturing said outer root sheath cells to obtain keratinocyte precursor cells;
 - (c) preparing an epidermal or dermal equivalent comprising said keratinocyte precursor cells; and
 - (d) applying a portion of said epidermal or ~~dermal~~ complex equivalent to said defect.
2. (Original) The method of claim 1, wherein said outer root sheath cells are autologous cells obtained from an individual who will subsequently undergo treatment for a skin defect.
3. (Original) The method of claim 1, wherein said outer root sheath cells are homologous cells.
4. (Currently Amended) The method of claim 1, wherein said epidermal or complex skin equivalent comprises outer root sheath cells cultured in a medium containing only homologous or autologous biological supplements.
5. (Currently Amended) The method of claim 1, wherein said epidermal or complex skin equivalent comprises outer root sheath cells cultured in a medium containing human serum in a concentration of less than 5%.
6. (Original) The method of claim 1, wherein the culture density of said keratinocyte precursor cells is between about 3×10^4 cells/cm² and about 1×10^5 cells/cm².

7. (Currently Amended) The method of claim 1, wherein said epidermal or complex skin equivalents are coated on their top or cornified side with a fibrin glue.

8. (Currently Amended) The method of claim 1, wherein said epidermal or complex skin equivalents are coated on their top or cornified side with a carrier membrane.

9. (Original) The method of claim 1, wherein the graft thickness for said epidermal equivalents is 50-150 microns.

10. (Original) The method of claim 1, wherein the graft thickness for said complex skin equivalents does not exceed 0.4 mm.

11. (Currently Amended) A method for the treatment of a skin defect comprising
- (a) culturing an intact hair follicle of an anagenic hair to obtain outer root sheath cells;
 - (b) culturing said outer root sheath cells to obtain keratinocyte precursor cells;
 - (c) preparing an epidermal or dermal equivalent comprising said keratinocyte precursor cells; and
 - (d) applying a portion of said epidermal or ~~dermal~~ complex skin equivalent to said defect

wherein all culturing of cells is performed in a medium which utilizes autologous or homologous human serum in a concentration of ~~preferably~~ less than approximately 5%.

12. (Original) The method of claim 11, wherein said anagen or growing hair is cultured *in toto*.

13. (Cancelled)

14. (Original) The method of claim 11, wherein said outer root sheath cells are homologous cells.

15. (Cancelled)

16. (Currently Amended) The method of claim 11, wherein said epidermal or complex skin equivalents are coated on their top or cornified side with a fibrin glue.

17. (Currently Amended) The method of claim 11, wherein said epidermal or complex skin equivalents are coated on their top or cornified side with a carrier membrane.

18. (Original) The method of claim 11, wherein the graft thickness for said epidermal equivalents is 50-150 microns.

19. (Original) The method of claim 11, wherein the graft thickness for said complex skin equivalents does not exceed 0.4 mm.

20. (Currently Amended) A method for the treatment of a skin defect comprising

- (a) culturing an intact hair follicle of an anagenic hair to obtain outer root sheath cells;
- (b) culturing said outer root sheath cells to obtain keratinocyte precursor cells;
- (c) preparing an epidermal or dermal equivalent comprising said keratinocyte precursor cells; and
- (d) applying a portion of said epidermal or ~~dermal~~ complex skin equivalent to said defect

wherein said epidermal or ~~dermal~~ complex skin equivalent is coated on its top or cornified side with a fibrin glue.

21. (Original) The method of claim 20, wherein said anagen or growing hair is cultured *in toto*.

22. (Original) The method of claim 20, wherein said outer root sheath cells are autologous cells obtained from an individual who will subsequently undergo treatment for a skin defect.

23. (Original) The method of claim 20, wherein said outer root sheath cells are homologous cells.
24. (Currently Amended) The method of claim 20, wherein said epidermal or complex skin equivalent comprises outer root sheath cells cultured in a medium containing only homologous or autologous biological supplements.
25. (Currently Amended) The method of claim 20, wherein said epidermal or complex skin equivalent comprises outer root sheath cells cultured in a medium containing human serum in a concentration of less than 5%.
26. (Original) The method of claim 20, wherein the culture density of said keratinocyte precursor cells is between about 3×10^4 cells/cm² and about 1×10^5 cells/cm².
27. (Currently Amended) The method of claim 20, wherein said epidermal or complex skin equivalents are coated on their top or cornified side with a fibrin glue which contains one or more anti-microbial, anti-fungal, or anti-viral agents emulsified therein.
28. (Currently Amended) A method for the selection of keratinocyte precursor cells from the outer root sheath of hair for subsequent use in a composition for healing a skin defect, comprising the steps of:
- (a) plucking of an intact anagen hair;
 - (b) primary-culturing the outer root sheath-derived keratinocyte precursor cells by adhering said intact anagen hair to a microporous membrane, which possesses growth-arrested/limited feeder cells on its undersurface so as to select for keratinocyte precursor cells from the outer root sheath of hair;
 - (c) organotypically-culturing the outer root sheath cells harvested from said primary cultures by inoculating a microporous membrane which also possesses growth-arrested/limited feeder cells on its undersurface;
 - (d) generating an epidermal or complex skin equivalent, for subsequent use as a graft insert, by placing a carrier membrane on top of said organotypic-culture from step

- (c) and detaching said complex skin or epidermal equivalent, which is comprised of the keratinocyte precursor cells and carrier membrane, together as a single, laminar unit;
 - (e) contacting said epidermal or complex skin equivalent with a skin defect present on an individual, and immobilizing said epidermal or skin equivalent at the site of contact.
29. (Original) The method of claim 28, wherein said outer root sheath cells are autologous cells derived from the individual who will subsequently undergo treatment for a skin defect.
30. (Original) The method of claim 28, wherein said outer root sheath cells are homologous cells.
31. (Original) The method of claim 28, wherein the culture density of said keratinocyte precursor cells is between about 3×10^4 cells/cm² and about 1×10^5 cells/cm².
32. (Original) The method of claim 28, wherein the culture density of said growth-arrested/limited feeder cells on said microporous membrane is between about 1×10^4 cells/cm² and about 5×10^4 cells/cm².
33. (Original) The method of claim 28, wherein said growth-arrested/limited feeder cells are banked or immortalized cells.
34. (Original) The method of claim 28, wherein said primary and organotypic cultures utilize autologous or homologous human serum.
35. (Original) The method of claims 28, wherein said primary and organotypic cultures utilize autologous or homologous human serum in a concentration of less than about 5%.

36. (Currently Amended) The method of claim 28, wherein said epidermal or complex skin equivalent comprises outer root sheath cells cultured in a medium containing only homologous or autologous biological supplements.

37. (Currently Amended) The method of claim 28, wherein said epidermal or complex skin equivalent comprises outer root sheath cells cultured in a medium containing only homologous or autologous releasates from blood components.

38. (Currently Amended) The method of claims 28, wherein said epidermal or complex skin equivalent comprises outer root sheath cells cultured in a medium containing only homologous or autologous releasates from blood components at a concentration of about 0.1% to about 20%.

39. (Original) The method of claim 28, wherein said epidermal equivalents are coated on their top or cornified side with a fibrin glue.

40. (Original) The method of claims 28, wherein said epidermal equivalents are coated on their top or cornified side with a carrier membrane.

41. (Original) The method of claims 28, wherein the graft thickness for said epidermal equivalents is 50-150 microns.

42. (Original) The method of claims 28, wherein the graft thickness for said complex skin equivalents does not exceed 0.4 mm.

43. (Original) The method of claim 28, wherein said microporous membrane is coated by one or ore extracellular matrix substances selected from a group consisting of: fibrin, fibronectin, collagens, laminins and hyaluronan.

44. (Original) The method of claims 28, wherein said microporous membrane possesses a growth-arrested/limited feeder cell system on its undersurface with said feeder cells of at least

one type of cell selected from the group comprising human dermal fibroblasts, epidermal cells, mesenchymal cells, neuronal cells and endothelial cells.

45. (Original) The method of claim 28, wherein said carrier membrane is made from one or more types of materials selected from the group comprising polyester, PTFE, polyurethane, hyaluronic acid, polylactic acid, collagen, or a silicone or vaseline gauze dressing.

46. (Original) The method of claim 28, wherein the size of said epidermal equivalent is selected from the group consisting of 1.0 cm, 1.5 cm, 2.0 cm, and 2.5 cm in diameter.

47. (Currently Amended) A method of shipping or transporting epidermal equivalents prepared according to the method of claim 1 comprising:

- (a) detaching said epidermal equivalents from a culture medium, and
- (b) transferring said epidermal equivalents onto a transport medium.

48. (Original) The method of claim 47, wherein said epidermal equivalents are coated on their top or cornified side with a carrier membrane.

49. (Original) The method of claim 48, wherein said epidermal equivalents are further sealed and shipped for future use in grafting.

50. (Original) The method of claim 47, wherein said transport medium comprises a solidified or gelled medium.

51. (Original) The method of claim 50, wherein said solidified or gelled medium is selected from the group consisting of agarose, methyl cellulose, or another gelifying substance.